25	GsC^{m}sC^{m}s C^{m}sAsAs GsC^{m}sTs GsGsC^{m}s ASTsC^{m}S C^{m}sGSTs C^{m}SA	human ICAM-1

[0279] All nucleosides in bold are 2=-O-(methoxyethyl); subscript s indicates a phosphorothioate linkage; underlined nucleosides indicate 2'-ara-(OH) modification. superscript m on C (Cm)indicates a 5-methyl-C.

Table XIV

Oligonucleotides Containing chimeric

2'-O-(2-methoxyethyl) and 2'-ara-(OMe) modifications

SEQ ID NO:	Sequence (5'-3')	Target
24	AsTsGs C^{m}sAsTs TsCs^{m}Ts GsCs^{m}Cs^{m} Cs^{m}C^{m}sC^{m}s AsAsGs GsA	mouse C-raf
25	GsC^{m}sC^{m}s C^{m}sAsAs GsC^{m}sTs GsGsC^{m}s ASTsC^{m}S C^{m}sGSTs C^{m}SA-3'	human ICAM-1

[0280] All nucleosides in bold are 2=-O-(methoxyethyl); subscript S indicates a phosphorothioate linkage; underlined nucleosides indicate 2'-ara-(OMe) modification. superscript m on C (C^{m})indicates a 5-methyl-C.

PROCEDURE 2

Enzymatic Degradation of 2'-O-modified oligonucleotides

[0281] Three oligonucleotides are synthesized incorporating the modifications shown in Table 2 below at the 3'-end. These modified oligonucleotides are subjected to snake venom

phosphodiesterase action.

[0282] Oligonucleotides (30 nanomoles) are dissolved in 20 mL of buffer containing 50 mM Tris-HCl pH 8.5, 14 mM $MgCl_2$, and 72 mM NaCl. To this solution 0.1 units of snake-venom phosphodiesterase (Pharmacia, Piscataway, NJ), 23 units of nuclease P1 (Gibco LBRL, Gaithersberg, MD), and 24 units of calf intestinal phosphatase (Boehringer Mannheim, Indianapolis, IN) are added and the reaction mixture is incubated at 37C for 100 hours. HPLC analysis is carried out using a Waters model 715 automatic injector, model 600E pump, model 991 detector, and an Alltech (Alltech Associates, Inc., Deerfield, IL) nucleoside/nucleotide column (4.6 x 250 mm). All analyses are performed at room temperature. The solvents used are A: water and B: acetonitrile. Analysis of the nucleoside composition is accomplished with the following gradient: 0-5 min., 2% B (isocratic); 5-20 min., 2% B to 10% B (linear); 20-40 min., 10% B to 50% B. The integrated area per nanomole is determined using nucleoside standards. Relative nucleoside ratios are calculated by converting integrated areas to molar values and comparing all values to thymidine, which is set at its expected value for each oligomer.

Table XV
Relative Nuclease Resistance of 2'-Modified Chimeric Oligonucleotides

5'-TTT TTT TTT TTT TTT T*T*T*T*-3' **SEQ ID NO: 26**
(Uniform phosphodiester)

T* = 2'-modified T
-S-Me
-Me
-2'-ara-(F)
-2'-ara-(OH)
-2'-ara-(OMe)

PROCEDURE 3

General procedure for the evaluation of chimeric C3'-endo and C2'-endo modified oligonucleotides targeted to *Ha-ras*

[0283] Different types of human tumors, including sarcomas, neuroblastomas, leukemias and lymphomas, contain active oncogenes of the *ras* gene family. *Ha-ras* is a family of small molecular weight GTPases whose function is to regulate cellular proliferation and differentiation by transmitting signals resulting in constitutive activation of *ras* are associated with a high percentage of diverse human cancers. Thus, *ras* represents an attractive target for anticancer therapeutic strategies.

[0284] SEQ ID NO: 27 (5'-TsCsCs GsTsCs AsTsCs GsCsTs CsCsTs CsAsGs GsG-3') is a 20-base phosphorothioate oligodeoxynucleotide targeting the initiation of translation region of human *Ha-ras* and it is a potent isotype-specific inhibitor of *Ha-ras* in cell culture based on screening assays (IC_{50}=45 nm). Treatment of cells *in vitro* with SEQ ID NO: 27 results in a rapid reduction of *Ha-ras* mRNA and protein synthesis and inhibition of proliferation of cells containing an activating *Ha-ras* mutation. When administered at doses of 25 mg/kg or lower by daily intraperitoneal injection (IP), SEQ ID NO: 27 exhibits potent antitumor activity in a variety of tumor xenograft models, whereas mismatch controls do not display antitumor activity. SEQ ID NO: 27 has been shown to be active against a variety of tumor types, including lung, breast, bladder, and pancreas in mouse xenograft studies (Cowsert, L.M. *Anti-cancer drug design*, **1997**, *12*, 359-371). A second-generation analog of SEQ ID NO: 27, where the 5' and 3' termini ("wings") of the sequence are modified with 2'-methoxyethyl (MOE) modification and the backbone is kept as phosphorothioate (Table XVI, SEQ ID NO: 27 (5'-TsCsCs GsTsCs AsTsCs GsCsTs CsCsTs CsAsGs GsG-3')), exhibits IC_{50} of 15 nm in cell culture assays. thus, a 3-fold improvement in efficacy is observed from this chimeric analog. Because of the improved nuclease resistance of the 2'-MOE phosphorothioate, SEQ ID NO: 27 (5'-TsCsCs GsTsCs AsTsCs GsCsTs CsCsTs CsAsGs GsG-3') increases the duration of antisense effect *in vitro*.